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A Coagulo-Flocculation Test for Malignant Tumors. (Studies on Antigens.)

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In these studies the following tissues were examined: wet human heart and liver, wet and dry beef heart, liver, kidney, brain and muscle. Three main types of antigens were employed:

Plain alcoholic extract. The wet tissues were extracted with 95% alcohol in the ratio 1:10 for 3 days at 37°C. and kept over night at room temperature. These extracts were then filtered. Dry tissues were extracted in the ratios 1:10, 1:25, 1:50, and 1:100 at 37°C., room and ice box temperature. The duration of extraction was the same as stated for the wet tissues.

Tractional extracts. They were prepared from the above dry tissues. Ten grams of the powderized tissues were extracted with 150 cc. of acetone and likewise with ether, chloroform, benzol and xylol. These flasks were kept at 37°C. for one hour, the extracts were then filtered and evaporated to dryness. The residue was redissolved in 50 cc. of 95% alcohol. Similarly extracts were prepared at ice box and at room temperature.

Secondary extracts. As in the preceding method 10 gm. of the respective dry tissues were extracted with 150 cc. of acetone for one hour at 37°C. and likewise with ether, chloroform, benzol and xylol. The extracts were then filtered and discarded, while the tissue residue was dried at 37°C. for one hour or for a longer time if necessary. Each dried tissue was extracted with 50 cc. of 95% alcohol for 3 days at 37°C. and then over night at room temperature. These extracts were again filtered. Similar extracts were prepared at ice box and at room temperature.

The usefulness of the above extracts with regard to the test can be summarized as follows: Beef heart appears to be the most suitable tissue for extraction of lipoids and is not exceeded in this respect by any other tissue. Wet beef heart is preferable to dry one, which renders a more concentrated extract, thus narrowing the specific zone. This can be partially overcome by extracting the dry beef heart with comparatively large amounts of lipoid solvents. By using the wet beef heart, the solvent becomes diluted by the tissue fluids and in this manner its extractive power becomes greatly

decreased. The secondary extracts do not possess any advantages over the plain extracts. The fractional extracts, which seem to be useful actually give the effect of the solvents, their specific zone being slightly narrowed by the lipoids they contain. The lipoids alone in the useful extracts cause such a turbidity in the tubes that even for this reason only their usefulness appears to be a very slight one.

Cholesterol lecithin and a number of alcohol soluble gums (copal, mastic, gamgabe, tragacanth, chicle, benzoin, damar, sandarc, shellac white and orange, elemi, guaiac, camphor, catechu and kino) were examined concerning their usefulness in place of lipoids or in combination with lipoids; their value was found to be very slight as compared with lipoids. Among the lipoid solvents ethyl-alcohol and methyl-alcohol were the most satisfactory ones. The influence of time and temperature of extraction is of far less importance in the extracts for malignant tumors than observed in various extracts for the Wassermann test or precipitation test for syphilis.

Ratio 1:10 *between tissue and the extracting fluid,* for wet beef heart and 95% alcohol appears to be the most satisfactory. The same ratio applies to plain alcoholic extracts from dry tissues made up at room or ice box temperature. For dry tissues extracted at 37°C. the best ratio was 1:100. Similar results were obtained with the secondary extracts: if the second extraction was carried out at 37°C., the best ratio was 1:100, while the extraction made at room temperature gave the best results in the ratio 1:10. The fractional extracts did not show much variation in titer, whether the lipoids were redissolved at room temperature or 37°C., whether the ratio was 1:10 or 1:100. The extracts are most satisfactorily preserved if they are kept in dark colored bottles in a dark place at a temperature not lower than that at which they were prepared.

As a result of these studies alcoholic beef heart extracts are found to be the most satisfactory ones. Their preparation is as follows: 1. Plain wet alcoholic beef heart extract is prepared by grinding fat free beef hearts and extracting them with 95% alcohol in the ratio 1:10 for 3 days at 37°C. and over night at room temperature and then filter. 2. Plain, dry alcoholic beef heart extract is prepared by extracting beef heart powder with 95% alcohol in the ratio 1:10 for 3 days at room temperature and then filter. Similar results were obtained if the extraction in the ratio 1:100 for 3 days at 37°C., overnight at room temperature and then filter.